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14. ABSTRACT

The study aims include investigating specific mechanisms of adverse effects related to RBC storage age in critically ill patients. IRB approvals for the clinical sites participating in this study have been approved, and patient enrollment began in March 2011. Thus far, we have enrolled 37 patients, from whom samples have been collected, processed, shipped from Canada to San Francisco. We have begun HLA typing and this has been completed for 10 patients' Day 0 samples so far. As there were delays in initiation of collecting patient samples, we have not begun coagulation and microparticle testing on the patient samples yet. Optimization work for the microparticle studies have built on related NIH-funded studies of in vitro properties of microparticles, refining the list of activation markers to be tested. The tests of patient samples will be performed in batches once enough samples have been obtained to minimize batch-to-batch variability in the testing results.

15. SUBJECT TERMS
RBC storage age; microchimerism; critically ill patients; coagulation; microparticles

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Adverse Effects of RBC Storage in Critically Ill Patients

INTRODUCTION

Combat casualties are specifically at risk of adverse effects resulting from the use of RBCs of increased storage age. A large multicenter randomized controlled trial in 30 Canadian centers of 2500 critically ill patients called the Age of Blood Evaluation (ABLE) trial has been funded. In this trial of critically ill patients, which includes patients with traumatic injuries, study groups will be randomized to either RBCs of < 8 days storage time or standard RBC storage time (mean 21 days). The primary outcome of this trial is 90 day mortality. Secondary outcomes include severity of multiple organ dysfunction syndrome, serious thrombotic events and nosocomial infections, and ICU and hospital length of stay. Prospective clinical studies investigating the mechanisms and clinical outcomes associated with increased or decreased RBC storage age in critically ill patients including traumatic injury have not been performed. The ABLE study presents a unique and probably one-time opportunity to investigate mechanisms in the context of clinical outcomes for well-characterized study groups. This study is designed to determine specific mechanisms of adverse effects related to the RBC storage age in transfused critically ill patients enrolled in the ABLE study. This ancillary study will specifically determine if the RBC unit storage time affects patient's immune function, inflammation, coagulation, microparticle concentrations and microchimerism.

Aims

1. To determine how RBC unit storage time affects coagulation in 100 critically ill patients, how these effects change over time after transfusion and if these parameters correlate with clinical outcomes.

1a. Quantify levels of Prothrombin Fragments 1+2, soluble Thrombomodulin, Protein C, PAI-1, tissue Plasminogen Activator, Factors V, VII, VIII, D-Dimer, Antithrombin III, soluble Endothelial Protein C Receptor, Xia, INR, PT & PTT using standard testing methods.

1b. Correlate patterns of measures of coagulation with receipt of blood stored for short vs. long periods.

1c. Correlate patterns of coagulation with inflammatory markers, immune function, microparticle concentration and clinical outcomes.

2. To determine if RBC unit storage time affects microparticle concentrations in both the RBC unit that is transfused and in 100 critically ill patients who are transfused. We will determine if microparticle concentrations correlate with coagulation, inflammation, altered immune function and clinical outcomes.

2a. Quantify the concentration of microparticles in the stored blood product from RBC unit segments and patient plasma before and after transfusion using Fluorescent Activated Cell Sorting (FACS) analysis.

2b. Define the likely cellular source of microparticles in the stored blood product and transfusion recipients using a panel of flow cytometry antibodies to define of the microparticles.

2c. Define the phenotype of microparticles in the stored blood product and transfusion recipients by staining them with panels of antibodies to activation markers.

2d. Correlate with systemic markers of coagulation, inflammation and immune dysfunction.

2e. Correlate microparticle concentration and activation marker profile with clinical outcomes.

3. To determine the incidence and magnitude of TA-MC in 200 critically ill patients.

3a. Determine the HLA type of transfusion recipients.

3b. Measure the presence of minor populations of non-self cells based on panels of insertion-deletion polymorphisms and HLA class II allele disparities using highly sensitive real-time Polymerase Chain Reaction (PCR) assays.

3c. Correlate TA-MC results with RBC storage age in addition to immune function and inflammation results from previously funded studies of the same patients enrolled in this study.

BODY

Research Ethics Board (REB) approvals were obtained for all the Canadian clinical sites, Blood Systems Research Institute (BSRI) through University of California San Francisco Institutional Review Board (UCSF IRB) and from Human Research Protection Office (HRPO). The manual of procedures was finalized and all site coordinators were informed of the processing methods. We began enrollment in March 2011. As of December 26th, 2011, we have enrolled a total of 37 patients in this study. Evaluable samples have been collected, processed and shipped from the clinical site to BSRI.

Aim 1: While patient samples have not yet been tested due to delays in collection of clinical samples, testing and optimization of the coagulation marker measurements has been ongoing. Using banked legacy samples as well as newly acquired patient samples from a related project in San Francisco we have been testing our functional coagulation assay equipment. These samples have provided reliable and reproducible data in similar and related projects and we anticipate no delays when samples are ready for batch testing during the next grant period. In addition to assay testing we have been working with our statistical and modeling group to outline and refine the modeling and analysis of the relationship between coagulation measures and defined outcomes which we expect to also begin during the upcoming period.

Aim 2: While patient samples have not yet been tested due to delays in acquiring the clinical samples, optimization of the microparticle (MP) panels has been performed. We have identified the size and density characteristics of MPs using TruCount beads (Fig. 1), allowing quantitative measurement of MPs in patient samples. The samples for Aims 1 and 2 are being stored at BSRI until they are ready to be analyzed. These tests will be performed in batches in order to avoid variability of test results due to testing procedures and/or reagents used.

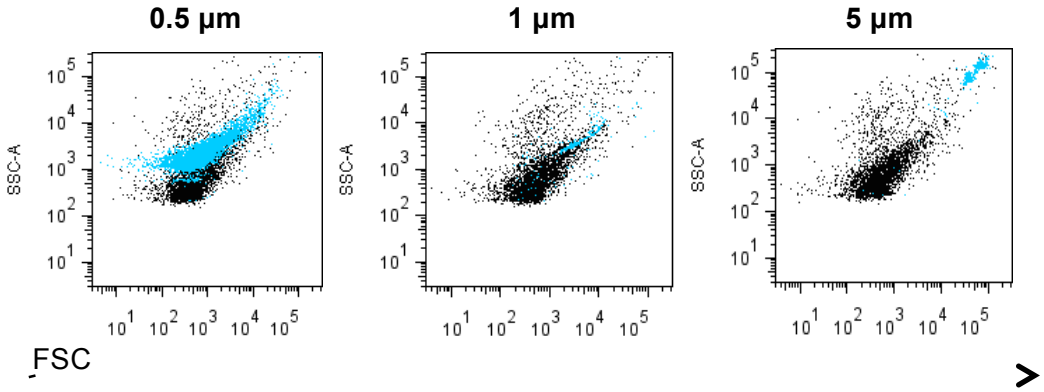

Figure 1. Validation of microparticle identification. The forward scatter (FSC, size) and side scatter (SSC, granularity) of MPs (black dots) was compared to TruCount beads (blue dots) of varied sizes from 0.5 to 5 µm. Purified MPs were identified in the 0.5 to 1 µm size range, consistent with expectations.

Aim 3: The first steps in microchimerism testing have been accomplished, namely defining the transfusion recipient alleles. We have performed HLA typing on 10 patients' Day 0 samples for which Day 0 and Day 28 (or Day 180) samples were available. These samples were typed for 12 Insertion/Deletions polymorphisms: SO1, SO3, SO4B, SO4, SO6, SO7, SO7B, SO8, SO8B, SO9, SO10 and SO11. There are 12 HLA-DRB1 low resolution types the samples were amplified for: 01, 03, 04, 07, 08, 09, 10, 11, 12, 13, 15 and 16. The next steps in measuring the presence of microchimerism will be to use real-time PCR to amplify the panel of alleles to detect minor, donor-derived populations longitudinally across each of the recipient's samples.

In order to increase the rate of enrollment we are currently in the process of enrolling another clinical site in Quebec, Canada. This will not affect the budget of the study, but will help meet enrollment targets. This has not been approved by HRPO yet, and will be submitted soon.

KEY RESEARCH ACCOMPLISHMENTS

- We have begun patient enrollment and sample collection at the clinical sites in Canada
- The samples are being processed, shipped and stored.
- To date, 37 patients have been enrolled in the study
- Samples will be tested in batches of 10 patients.
- Day 0 samples for 10 patients have been HLA typed

REPORTABLE OUTCOMES

As mentioned above, we have begun sample acquisition. We have started HLA typing as the first step towards determining the incidence of microchimerism. Sample collection continues and samples will be tested in batches.

CONCLUSION

The ABLE ancillary study has launched and is accruing patients at the rate expected. In addition to managing current study accrual, new sites are actively being explored to enhance the pace of subject enrolment. The sample repository is being maintained, and coagulation, microparticle and microchimerism analyses will be performed when a sufficient sample size has accrued to allow batch processing, to maximize the quality of the data generated. HLA typing for Day 0 samples for microchimerism testing has been performed for 10 patients.

REFERENCES

None

APPENDICES

None